(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 451 824 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **91105704.0**

(22) Date of filing: **10.04.91**

(51) Int. Cl.5: **C07C 311/36**, C07C 229/76, C07C 229/26, A61K 49/02, A61K 49/00, A61K 43/00

(30) Priority: **10.04.90 JP 94353/90**

(43) Date of publication of application: **16.10.91 Bulletin 91/42**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB IT LI LU NL SE**

(71) Applicant: **NIHON MEDI-PHYSICS CO., LTD.**
9-8, Rokutanji-cho, Nishinomiya
Hyogo(JP)

(72) Inventor: **Seri, Shigemi**
Sumitomo Kagaku Sodegaura Shataku 1-204
98 Daijuku
Sodegauramachi, Kimitsu-gun, Chiba(JP)
Inventor: **Yamauchi, Hirohiko**
Sumitomo Kagaku Anegasaki Shataku 825
1-9, Yushudainishi, Ichihara Chiba(JP)
Inventor: **Azuma, Makoto**
750, Saihiro
Ichihara, Chiba(JP)
Inventor: **Arata, Yoji**
c/o Urban Heights Kitasuna 1122
5-20-18, Kitasuna, Koto-ku, Tokyo(JP)

(74) Representative: **Vossius & Partner**
Siebertstrasse 4 P.O. Box 86 07 67
W-8000 München 80(DE)

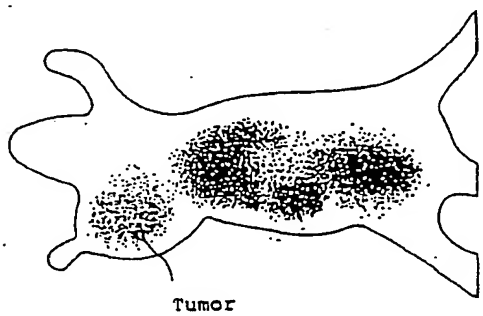
(54) Chelating compounds and their use.

(57) A chelating compound of the formula:

$(R\text{-}NHOC\text{-}CH_2)_n\text{-}A\text{-}(CH_2COOH)_m$ (I)

wherein R is an aromatic ring-containing organic group, A is a residue of an aminopolyacetic acid excluding acetic acid groups ($-CH_2COOH$) therefrom, m is an integer of at least two and n is an integer of 1 or 2, or its salt, which has a specificity to a hepatobiliary system so that a chelate compound formed between said chelating compound and a metallic element through a chelate bond is useful as a diagnostic or therapeutic agent for hepatobiliary organs and tissues.

Fig. 1: Scintigram of hepatoma-transplanted rat using In-111-5-DNS-etn-DTPA (70 hours after administration)



EP 0 451 824 A2

The present invention relates to chelating compounds and their use. More particularly, it relates to chelating compounds having a chelate-forming property and a specificity or selectivity to a hepatobiliary system, and their use as carriers far metal elements suitable for diagnosis or therapy of hepatobiliary organs or tissues.

In recent years, the number of patients suffering from diseases in a hepatobiliary system such as hepatoma have significantly increased. It is, therefore, highly desirable to establish a reliable diagnostic method, particularly through imaging, as well as an effective therapeutic method.

Among various imaging agents for hepatobiliary organs or tissues as heretofore reported, there is known technetium-99m-N-pyridoxyl-5-methyltryptophan (Tc-99m-PMT). Imaging with this chelate compound is well evaluated in showing a significant specificity to hepatocellular carcinoma (Hasegawa et al.: Cancer, 57, 230-236 (1986)). Unfortunately, however, its sensitivity is somewhat low, i.e. 60 %. Diethylenetriaminepentaacetato gadolinium (Gd-DTPA) is also known as a nuclear magnetic resonance (NMR) imaging agent which can provide useful information for the diagnosis of abdominal organs (Weinmann et al.: AJR, 142, 619-629 (1984)). However, it is excreted into urine so quickly that its distribution in liver is insufficient and satisfactory informations on liver are hardly obtainable.

As well known, aminopolycarboxylic acids have an excellent chelate-forming property and are useful as carriers for metallic elements suitable for diagnosis. Thus, the chelate compounds formed between aminopolycarboxylic acids and metallic elements are used as imaging agents for radioactive diagnosis, nuclear magnetic resonance (NMR) diagnosis, etc.

It has now been unexpectedly found that the introduction of a certain aromatic ring-containing organic group into an aminopolycarboxylic acid is effective in enhancing the specificity or selectivity to a hepatobiliary system. When, for instance, DTPA (diethylenetriaminepentaacetic acid) is administered intravenously into a mammalian body, it is mainly excreted into urine. In contrast, it was experimentally confirmed that N,N''-bis-[(2-dansylaminoethyl)carbamoylmethyl]-diethylene-triamine-N,N',N''-triacetic acid (B-DNS-etn-DTPA) obtained by introducing two dansyl groups into DTPA is excreted mainly into the intestine through a hepatobiliary system.

As stated above, aminopolycarboxylic acids are well known chelating compounds. Since the chelate bond formed between aminopolycarboxylic acids and metallic elements are generally stable in a mammalian body or at a physiological pH range, they are practically used as carriers for metallic elements to make imaging agents, for instance, Gd-DTPA as above mentioned. However, it has never been known that their specificity or selectivity to a hepatobiliary system is significantly enhanced by introducing a certain aromatic ring-containing organic group therein.

The present invention is based on the above finding and provides a chelating compound which has a high specificity or selectivity to a hepatobiliary system and is useful as a carrier for a metallic element to give a diagnostic or therapeutic agent for hepatobiliary organs and tissues.

The chelating compound of the invention is an aminopolycarboxylic acid, particularly an aminopolyacetic acid, in which one or two carboxylic groups are each combined with an aromatic ring-containing organic group, particularly through a carbonamide (-CONH-) linkage and at least two carboxylic groups are each kept in a free or salt form to have a chelate-forming property with a metallic element.

More specifically, the chelating compound is represented by the formula:

$$(R\text{-}NHOC\text{-}CH_2)_n\text{-}A\text{-}(CH_2\text{-}COOH)_m \quad (I)$$

wherein R is an aromatic ring-containing organic group, A is a residue of an aminopolyacetic acid excluding acetic acid groups ($-CH_2COOH$) therefrom, m is an integer of at least two and n is an integer of at least one. The carboxyl groups therein may be in a free or salt form.

The aminopolyacetic acid comprises a hydrocarbon chain in a straight, branched or cyclic form, at least two amino groups present in the hydrocarbon chain (such as -C-NH-C-) and/or at the end of the hydrocarbon chain (such as $-C-NH_2$) and at least three acetic acid groups ($-CH_2-COOH$) each attached to a nitrogen atom in said amino groups. Specific examples or the aminopolyacetic acid are ethylenediaminetetraacetic acid (EDTA), diethylenetriamine-pentaacetic acid (DTPA), trans-1,2-cyclohexadiamine-tetraacetic acid (CyDTA), 1,4,7,10-tetraazacyclododecane-tetraacetic acid (DOTA), etc. As to other specific examples of the aminopolyacetic acid, reference may be made to JP-A-58/29718 (DE-A-3129906).

In the chelating compound of the invention, at least two acetic acid groups originated from the aminopolyacetic acid are kept in a free form or in a salt form such as an alkali metal salt so as to capture a metallic element through a chelate bond, while at least one acetic acid group is combined with the aromatic ring-containing organic group. Preferably, the aromatic ring-containing organic group is originated from an aromatic ring-containing organic amine, and the combination between the aminopolyacetic acid and the

aromatic ring-containing organic amine is made through a carbonamide (-CONH-) linkage.

As the aromatic ring-containing organic group, there are exemplified aryl, ar(lower)alkyl, arylsulfonyl, ar-(lower)alkylsulfonyl, arylamino(lower)alkyl, ar(lower)alkylamino(lower)alkyl, arylsulfonylamino(lower)alkyl, ar-(lower)alkylsulfonylamino(lower)alkyl, etc. The aryl portion, of which examples are phenyl, naphthyl, etc., in these groups may be optionally substituted with lower alkyl (e.g. methyl, ethyl), lower alkylamino (e.g. methylamino, ethylamino), di(lower)alkylamino (e.g. dimethylamino, diethylamino), etc. Thus, specific examples of the aromatic ring-containing organic group represented by the symbol R are phenyl, lower alkylphenyl such as tolyl (e.g. p-tolyl), di(lower)alkylaminophenyl such as dimethylaminophenyl (e.g. p-dimethylaminophenyl), phenyl(lower)alkyl such as phenethyl, benzenesulfonylamino(lower)alkyl such as benzenesulfonylaminoethyl, lower alkylbenzenesulfonylamino(lower)alkyl such as toluenesulfonylaminoethyl (e.g. p-toluenesulfonylaminoethyl), di(lower)alkylaminonaphthalenesulfonylamino(lower)alkyl such as dimethylaminonaphthalenesulfonylaminoethyl or dimethylaminonaphthalenesulfonylaminohexyl, naphthylamino(lower)alkyl such as naphthylaminoethyl, naphthyl(lower)alkyl such as naphthylmethyl, naphthalenesulfonylamino(lower)alkyl such as naphthalenesulfonylaminoethyl, etc., among which naphthyl-(lower)alkyl, naphthylamino(lower)alkyl, naphthalenesulfonylamino(lower)alkyl, 5-dimethylaminonaphthalene-1-sulfonylamino(lower)alkyl (i.e. dansylamino(lower)alkyl), etc. are favorable.

Production of the chelating compound of the invention nay be achieved by a per se conventional procedure for formation of a carbonamide linkage between an amino group and a carboxyl group, for instance, reacting an aromatic ring-containing organic amine of the formula: $R\text{-}NH_2$ (wherein R is as defined above) with an aminopolyacetic acid of the formula: $(HOOCCH_2)_n\text{-}A\text{-}(CH_2COOH)_m$ (wherein A, m and n are each as defined above) in any reactive form. The reaction may be carried out usually in an inert solvent (e.g. tetrahydrofuran, dioxane, dimethylformamide, benzene, toluene), if necessary, in the presence of a condensing agent such as a base, a dehydrating agent or the like. Depending on the reaction conditions, particularly the proportion of the aromatic ring-containing organic amine to the aminopolyacetic acid, there is produced the objective chelating compound having one or two aromatic ring-containing organic groups as the major product. When their mixture is obtained, the mono-substituted product and the bis-substituted product can be easily separated by a per se conventional separation procedure such as chromatography. In general, the bis-substituted product is favorable, because of its higher specificity or selectivity to a hepatobiliary system.

The thus obtained chelating compound can be converted into the corresponding chelate compound by treatment with a metal element in a per se conventional procedure for chelate-formation. The kind of the metal element may be appropriately chosen depending on the purpose for which the chelate compound is used.

For the nuclear medicine such as nuclear diagnosis or nuclear therapy, various radioactive metal elements may be used. For instance, the use of such gamma-ray emitting metal elements as technetium-99m, indium-111 and gallium-67 are preferred in order to produce tumor-imaging agents. On the other hand, beta-ray emitting metal elements such as rhenium-186, rhenium-188 and yttrium-90 are clinically useful for treatment of tumors.

For instance, B-DNS-etn-DTPA as an example of the invention is promptly excreted from the normal or healthy liver to bile ducts, but when a tumor is present in liver, it has difficulty excreting into the bile ducts because no efficient bile duct exists in the tumor portion. Utilizing this dynamic behavior, a chelate complex of B-DNS-etn-DTPA with indium-111 is used as a radioactive imaging agent for diagnosis of a hepatobiliary system, and a chelate complex of B-DNS-etn-DTPA with rhenium-186 may be employed to irradiate the tumor portion in liver for the therapeutic purpose.

Metallic elements usable for NMR imaging are required to be paramagnetic, and their preferred examples are lanthanoid elements under Atomic Nos. 57 to 70 and transition metal atoms under Atomic Nos. 21 to 29, 42 and 44. Among them, gadolinium, dysprosium, etc. are especially preferred because of their strong magnetic moment and chemical stability. These paramagnetic metallic elements are often toxic in concentrations required for NMR imaging, and therefore their amounts to be introduced into mammalian bodies are desired to be kept as small as possible. The administration of those paramagnetic metallic elements in the form of chelate complexes with the chelating compounds of the invention is quite advantageous, because the toxicity of the metallic elements is suppressed by the chelate formation and also their amounts to be administered for effective NMR imaging are lowered due to their specificity assuring an efficient accumulation at the target organ or tissue in a hepatobiliary system.

For instance, diethylenetriamine- pentaacetato gadolinium (III) (Gd-DTPA) is normally administered in the clinical use by intravenous injection at a dose of 100 μmol/kg. Since, however, its distribution is not specific to a hepatobiliary system, the excretion into urine is made promptly. As the result, sufficient contrast useful for diagnosis can be obtained only over a period of time producing the differences in

JOCID: <EP 0451824A2 I >

concentration among tissues or organs. In fact, the administation of Gd-DTPA to rats at a dose of 50 μmol/mg does not produce any change of signal intensity in liver (Kawamura et al.: Image Information, 21, 206-207 (1989)). Administration of a chelate complex of Gd(III) with N-[(2-dansylaminoethyl)-carbamoylmethyl]-diethylenetriamine-N,N',N'',N''-tetraacetic acid (Gd(III)-DNS-etn-DTPA) to rats produces enhancement of the T_1 relaxation in liver even at such a small dose as 20 μmol/kg, and this effect remains for one hour after the administration. Thus, Gd(III)-DNS-etn-DTPA is specifically taken up into the liver so that satisfactory NMR imaging can be obtained even at a low dose.

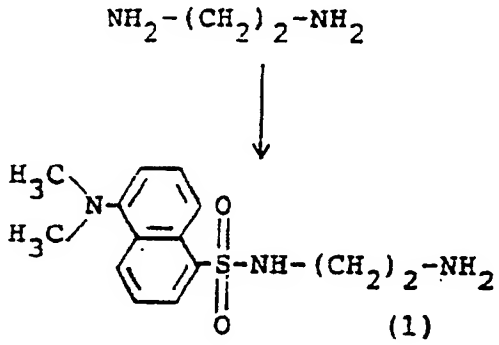
When the use for X-ray diagnosis is aimed at, the chelating compound of the invention may be complexed with a metallic element from Atomic Nos. 57 to 83, particularly lanthanum to form a chelate compound.

Practical and presently preferred embodiments of the invention are illustratively shown in the following Examples.

Example 1

Preparation of N-[(2-dansylaminoethyl)carbamoylmethyl]-diethylenetriamine-N,N',N'',N''-tetraacetic acid (DNS-etn-DTPA)(2) and N,N''-bis[(2-dansylaminoethyl)carbamoylmethyl]-diethylenetriamine-N,N',N''-triacetic acid (B-DNS-etn-DTPA (3):-

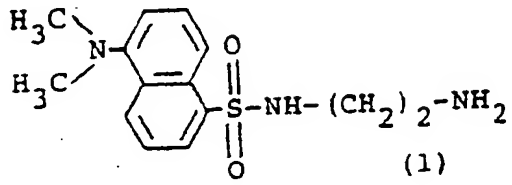
A. N-Dansyl-ethylenediamine (1)



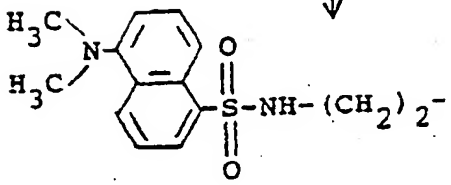
(1)

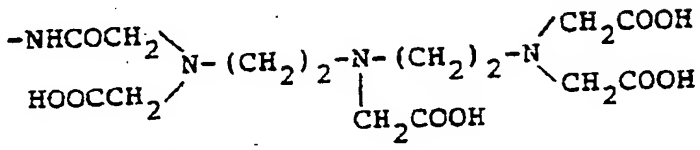
To a solution of ethylenediamine (635 mg, 10.6 mmol) in chloroform (10 ml), a solution of dansyl chloride (285 mg, 1.06 mmol) in chloroform (12 ml) was portionwise added, and the resultant mixture was stirred at room temperature overnight, followed by addition of a small amount of 1 N sodium hydroxide thereto for hydrolysis of unreacted dansyl chloride. The reaction mixture was concentrated, and the residue was combined with acetone. Insoluble materials were removed by filtration, and the filtrate was concentrated. Water (50 ml) was added to the residue, which was then extracted With ethyl acetate three times. The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was dissolved in a small amount of ethyl acetate, a small amount of n-hexane was added thereto, and the resultant mixture was allowed to stand at room temperature overnight. The precipitated crystals were collected and recrystallized from ethyl acetate to give Compound (1) (124 mg). Yield, 63 %.

B. DNS-etn-DTPA (2) and B-DNS-etn-DTPA (3)



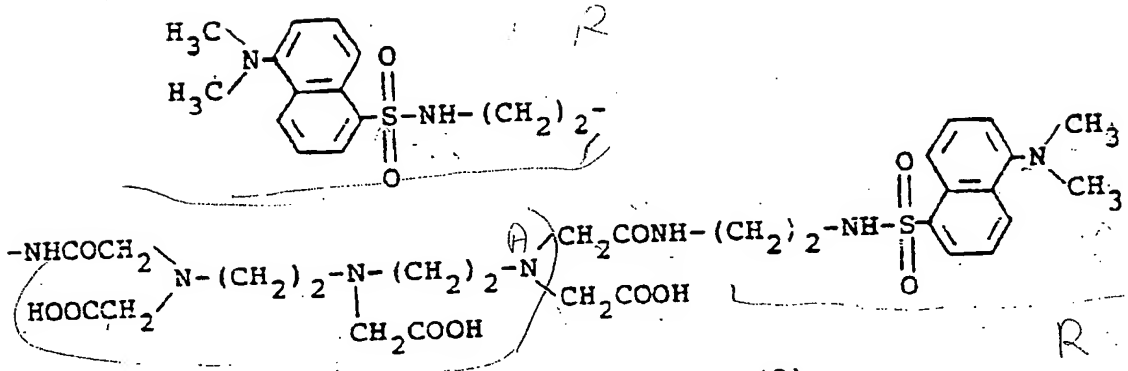
(1)





(2)

+



(3)

Diethylenetriamine-N,N,N',N''N''-pentaacetic acid anhydride (DTPA)1.39 g, 3.89 mmol) was dissolved in dimethylformamide (30 ml) under heating, and the resultant solution was cooled to room temperature. A solution of Compound (1) (113 mg, 0.385 mmol) in dimethylformamide (5 ml) was portionwise added thereto while stirring, and stirring was continued at room temperature for 1.5 hours. After concentration, the residue was combined with 0.1 M carbonate buffer (pH, 9.0, 20 ml) and subjected to anionic resin exchange chromatography (resin: AG-X4; eluting solution: 0.3 - 3M formic acid) and thin layer chromatography (support: silica gel 60; developing solvent: ethanol/aqueous ammonia = 4/1) for purification, whereby Compound (2) (69 mg) and Compound (3) (72 mg) respectively in yields of 27 % and 20 % were obtained.

Compound (2):-

IR (KBr) cm^{-1}: SO_2-NH (1140, 1320), COO^- (1400, 1590), CO-NH (1660, 3420), $C_{10}H_6$-N-$(CH_3)_2$ - (2800), CH_2 (2950).

FAB-MS (negative):$(M-H)^-$ (667 m/z), $(M+Na-2H)^-$ (689 m/z), $(M+2Na-3H)^-$ (711 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{28}H_{38}N_6O_{11}S_1Na_2.4\frac{1}{2}H_2O$.

Compound (3):-

IR (KBr) cm^{-1}: SO_2-NH (1140, 1320), COO^- (1410, 1590), CO-NH (1660, 3400), $C_{10}H_6$-N-$(CH_3)_2$ - (2800), CH_2 (2950).

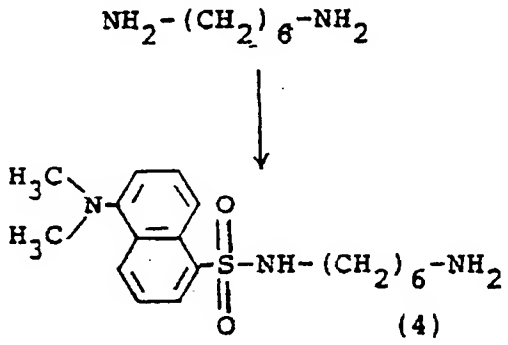
FD-MS: (M + H) (945 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{42}H_{54}N_9O_{12}S_2Na_3.8H_2O$.

Example 2

Preparation of N-[(6-dansylaminohexyl)carbamoylmethyl]-diethylenetriamine-N,N',N'',N''-tetraacetic acid (DNS-hxn-DTPA)(5) and N,N''-bis[(6-dansylaminohexyl)carbamoylmethyl]-diethylenetriamine-N,N',N''-triacetic acid (B-DNS-hxn-DTPA)(6):-

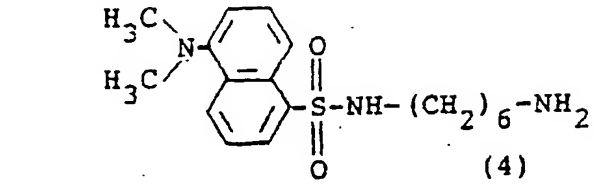
A. N-Dansyl-hexamethylenediamine (4)



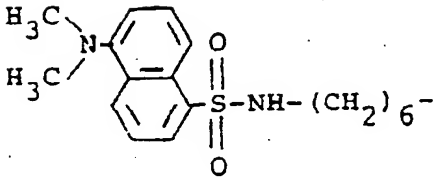
(4)

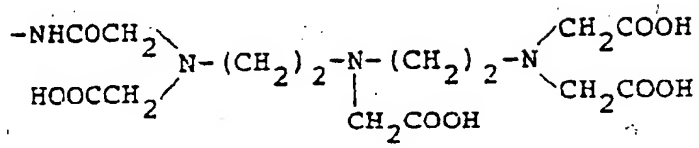
Hexamethylenediamine (5.39 g, 45.9 mmol) was combined with dimethylformamide (15 ml), and a solution of dansyl chloride (2.40 9, 8.7 mmol) in dimethylformamide (10 ml) was portionwise added thereto, followed by stirring at room temperature for 4 hours. Insoluble materials were removed by filtration, and the filtrate was stirred at room temperature overnight. After concentration, water and ethyl acetate were added thereto, and further 1N hydrochloric acid was added thereto to adjust the aqueous layer to pH 4. The aqueous layer was extracted with ethyl acetate three times, adjusted to pH 11 with potassium carbonate and extracted with ethyl acetate two times. The extracts were combined together, washed with water three times, dried over anhydrous sodium sulfate and concentrated to give Compound (4) (1.04 g) as an oil. Yield, 34 %.

B. DNS-hxn-DTPA (5)



(4)





(5)

DTPA anhydride (777 mg, 2.18 mmol) was dissolved in dimethylformamide (20 ml) under heating, and the resultant solution was cooled to room temperature. A solution of compound (4) (130 mg, 0.372 mmol) in dry dimethylformamide (5 ml) was portionwise added thereto while stirring, and stirring was continued at room temperature for 1 hour. After concentration, the residue was combined with 1M carbonate buffer (pH, 9.0, 50 ml) and allowed to stand in a refrigerator overnight Insoluble materials were removed by filtration, and the filtrate was treated in the same manner as in Example 1 B. to give Compound (5) (47 mg). Yield, 17%.

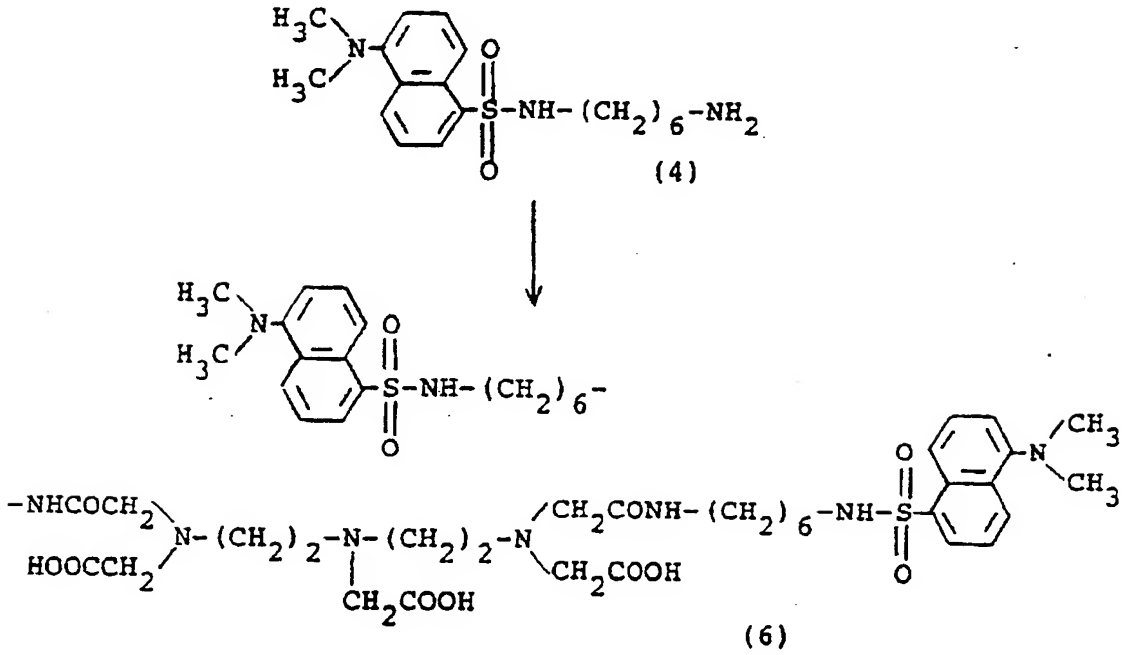
Compound (5):-

IR (KBr) cm^{-1}: SO_2-NH (1140, 1320), COO^- (1410, 1590), CO-NH (1660, 3420), $C_{10}H_6$-N-$(CH_3)_2$ - (2800), CH_2 (2950).

FAB-MS(negative): $(M+Na-2H)^-$ (745 m/z), $(M+K-2H)^-$ (761 m/z), $(M+2Na-3H)^-$ (767 m/z), $(M+Na+K-3H)^-$ (783 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{32}H_{46}N_6O_{11}S_1Na_2.6H_2O$.

C. B-DNS-hxn-DTPA (6)



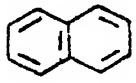
(4)

(6)

The insoluble materials as removed in the above B were collected and dissolved in methanol. The resultant solution was concentrated, and the residue was subjected to thin layer chromatography for purification, whereby Compound (6) (24 mg) was obtained. Yield, 6 %.

Compound (6):-

IR (KBr) cm^{-1}: SO_2-NH (1140, 1310), COO^- (1400, 1580), CH_2 (1450, 2930),



(1500, 3070), CO-NH (1660, 3400), $C_{10}H_6$-N-$(CH_3)_2$ (2780).

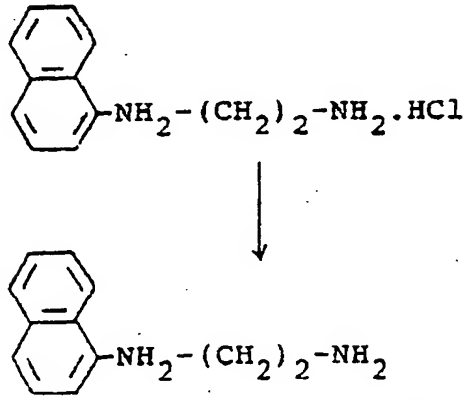
FAB-MS (negative): $(M-H)^-$ (1054 m/z), $(M+Na-2H)^-$ (1076 mz/), $(M+K-2H)^-$ (1092 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{50}H_{71}N_9O_{12}S_2Na_2.7H_2O$.

Example 3

Preparation of N-[[2-(1-naphthylamino)ethyl]carbamoylmethyl]-diethylenetriamine-N,N',N'',N''-tetraacetic acid (8):-

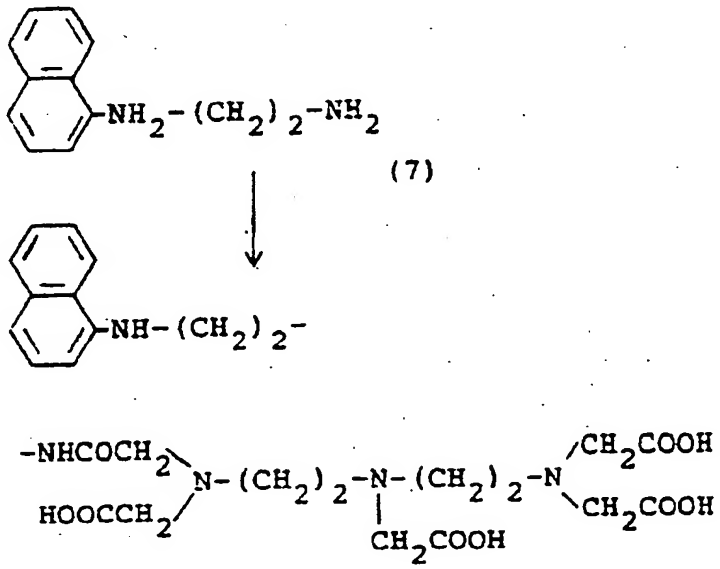
A. N-1-Naphthylethylenediamine (7)



(7)

To a suspension of N-1-naphthylethylenediamine dihydrochloride (746 mg, 2.88 mmol) in chloroform (50 ml), a saturated solution of sodium hydrogen carbonate (50 ml) was added, and the resultant mixture was stirred. The organic layer was collected, washed with a saturated solution of sodium chloride two times, dried over anhydrous sodium sulfate and concentrated to give Compound (7) (310 mg) as an oil. Yield, 58 %.

B. N-[[2-(1-Naphthylamino)ethyl]carbamoylmethyl]-diethylenetriamine-N,N',N",N"-tetraacetic acid (8)



(8)

DTPA anhydride (2.02 g, 5.60 mmol) was dissolved in dimethylformamide (20 ml) under heating and cooled to room temperature. A solution of Compound (7) (218 mg, 1.17 mmol) in acetone (5 ml) was added thereto while stirring, and stirring was continued at room temperature for 1.5 hours. The resultant mixture was allowed to stand at room temperature in a dark place overnight. The reaction mixture was treated with active carbon and concentrated. To the residue, 0.1 M carbonate buffer (pH, 8.9, 20 ml) was added, and the resultant mixture was treated with active carbon, followed by concentration. The residue was dissolved in

0.1 M carbonate buffer (pH, 8.9, 15 ml), treated with active carbon and subjected to anionic exchange resin chromatography (resin: AG-X4, eluting solution: 1.2 - 4.8 M formic acid) and thin layer chromatography to give Compound (8) (34 mg). Yield, 5 %.

Compound (8):-

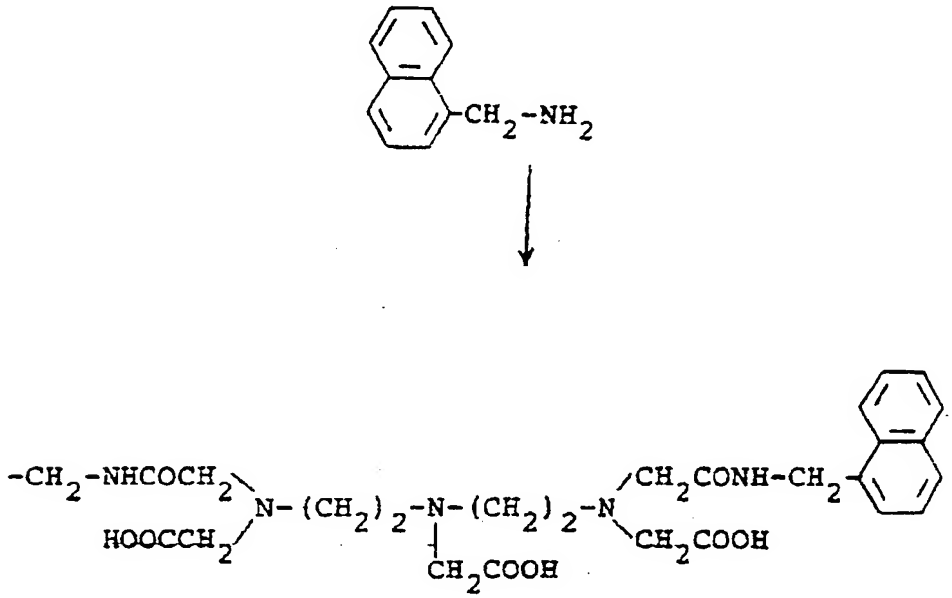
IR (KBr) cm^{-1}: COO^- (1400, 1580), CO-NH (1660, 3400), CH_2 (2960).

FAB-MS (positive): $(M+2Na-H)^+$ (606 m/z), $(M+K+Na-H)^+$ (622 m/z), $(M+4Na-3H)^+$ (650 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{26}H_{33}N_5O_9Na_2.6H_2O$.

Example 4

Preparation of N,N''-bis(1-naphthylmethylcarbamoylmethyl)-diethylenetriamine-N,N',N'',N''-triacetic acid (9):-

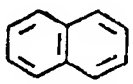


(9)

DTPA anhydride (2.05 g, 5.74 mmol) was dissolved in dimethylformamide (20 ml) while hot and cooled to room temperature. A solution of 1-nathphalenemethylamine (467 mg, 2.88 mmol) in acetone (5 ml) was portionwise added thereto, and the resultant mixture was stirred at room temperature for 2 hours. After concentration, 0.1 M carbonate buffer (pH 8.9, 30 ml) was added to the residue. Insoluble materials were collected, dissolved in methanol under heating and then cooled. After removal of insoluble materials by filtration, the filtrate was concentrated. The residue was dissolved in a small amount of dimethylformamide and subjected to thin layer chromatograph for purification, whereby Compound (9) (114 mg) was obtained. Yield, 6 %.

Compound (9):-

IR (KBr) cm^{-1}: COO^- (1400, 1590),



(1510, 3050), CO-NH (1650, 3400), CH_2 (2950).

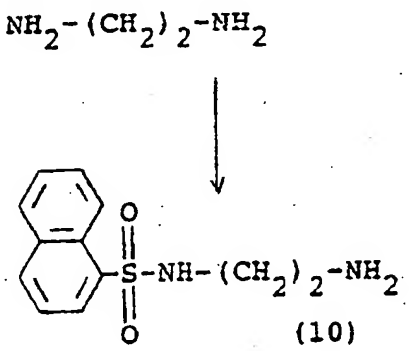
FAB-MS (positive): $(M+K)^+$ (710 m/z), $(M+K+Na-H)^+$ (732 m/z) , $(M+2K-H)^+$ (748 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{36}H_{40}N_5O_8Na_1.5H_2O$.

Example 5

Preparation of N-[[2-(1-naphthalenesulfonylamino)ethyl]carbamoylmethyl]-diethylenetriamine-N,N',N'',N''-tetraacetic acid (11):-

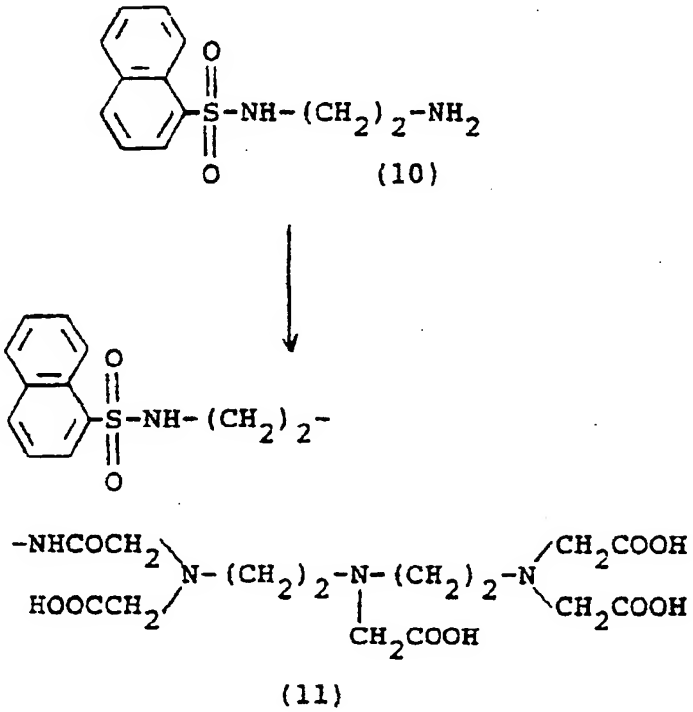
A. N-(1-Naphthalenesulfonyl)-ethylenediamine (10)



(10)

To a solution of dry ethylenediamine (1.06 g, 17.6 mmol) in dimethylformamide (10 ml), triethylamine (1.79 g, 17.7 mmol) was added, followed by stirring. While stirring at room temperature, a solution of 1-naphtalenesulfonyl chloride (4.00 g, 17.6 mmol) in dimethylformamide (15 ml) was portionwise added thereto, and stirring was continued for 1 hour under ice-cooling. Insoluble materials were removed by filtration, and the filtrate was concentrated. To the residue, chloroform and water were added, and insoluble materials were eliminated by filtration. From the filtrate, the aqueous layer was collected, washed with ethyl acetate two times and adjusted to pH 11 with potassium carbonate, followed by extraction with ethyl acetate three times. The extracts were combined, washed with water two times, dried over anhydrous sodium sulfate and concentrated. The residue was allowed to stand in a dark place overnight, and the precipitated crystals were collected and recrystallized From ethyl acetate to give Compound (10) (153 mg). Yield, 4 %.

B. N-[[2-(1-Naphthalenesulfonylamino)ethyl]-carbamoylmethyl]-diethylenetriamine-N,N',N",N"-tetraacetic acid (11)



(10)

(11)

DTPA anhydride (996 mg, 2.79 mmol) was dissolved in dimethylformamide (20 ml) while hot, and the resultant solution was cooled to room temperature. A solution of Compound (10) (139 mg, 0.557 mmol) in acetone (20 ml) was portionwise added thereto at room temperature under vigorous stirring. After completion of the addition, the resultant mixture was stirred at room temperature for 1 hour and then allowed to stand overnight. After concentration, the residue was dissolved in 0.1 M carbonate buffer (pH 8.9, 15 ml) and then treated in the same manner as in Example 1 B to give Compound (11) (66 mg). Yield, 19 %.

Compound (11):-

IR (KBr) cm^{-1}: SO_2-NH (1160, 1320), COO^- (1400, 1590), CH_2 (2870), CO-NH (3420).

FAB-MS (negative): $(M+Na-2H)^-$ (646 m/z), $(M+K+2H)^-$ (662 m/z), $(M+2Na-3H)^-$ (668 m/z), $(M+Na+K-3H)^-$ (684 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{26}H_{34}N_5O_{11}S_1Na_1.6H_2O$.

As understood from the results of the elementary analysis as above, the products in Examples 1 to 11, i.e. Compounds (2), (3), (5), (6), (8), (9) and (11), were obtained in the form of sodium salt. This is probably due to the support used in thin layer chromatography at the stage of purification.

Example 6

In-111-DNS-etn-DTPA (complex):-

A. Preparation of In-111 complex with Compound (2)

Compound (2) (0.93 mg, 1.39 μmol) was dissolved in 0.2 M acetate buffer (pH 5.3, 0.46 ml), and a 0.2 M acetate buffer solution (pH 5.3, 0.46 ml) containing indium chloride (^{111}In, 69.1 MBq) was added thereto. The resultant mixture was shaken for 30 seconds to give In-111-DNS-etn-DTPA.

B. Behavior of In-111-DNS-etn-DTPA on thin layer chromatography

An appropriate amount of In-111-DNS-etn-DTPA was spotted onto a silica gel plate (silica gel 60 manufactured by Merck Co., Ltd.) at a distance of 2 cm from the bottom and developed for 10 cm using a mixture of methanol-acetic acid (5 : 3) as a developing solvent. After air-drying, the plate was scanned with a thin layer radiochromatoscanner (Aloca Co.) to determine the distribution of radioactivity, and the radiochemical purity was calculated with a data processing apparatus (D-2000, manufactured by Hitachi Ltd.).

As the result, a single radioactivity peak (Rf = 0.13) was observed. Since the Rf value of this peak is different from that (Rf = 0) of indium acetate (^{111}In) or indium chloride (^{111}In) as a possible radiochemical impurity, the radiochemical purity of In-111-DNS-etn-DTPA was determined to be 100 %.

C. Behavior of In-111-DNS-etn-DTPA on electrophoresis

An appropriate amount of In-111-DNS-etn-DTPA was spotted on an acetylated cellulose membrane and subjected to electrophoresis using 50 mM phosphate buffer (pH 7.4) with a constant current of 1 mA/cm at room temperature for 30 minutes. In the same manner as in the above B, the membrane was scanned with a thin layer radiochromatoscanner to determine the distribution of radioactivity. As the result, it was revealed that In-111-DNS-etn-DTPA is a complex having a single negative charge.

Example 7

Other In-111 complexes:-

In the same manner as in Example 6 A, B and C, In-111 complexes with Compounds (3), (5), (6), (8), (9) and (11) were prepared, and their behaviors on thin layer chromatography (TLC) and electrophoresis (EP) as well as their radiochemical purity were determined. The results are shown in Table 1.

Table 1

Carrier compound	TLC (Rf)	EP	Radiochemical purity (%)
(3)	0.12	-	100
(5)	0.14	Negative	100
(6)	0.08	-	100
(8)	0.19	Negative	100
(9)	0.20	Positive	100
(11)	0.24	Negative	100

Example 8

Gd-DNS-etn-DTPA (complex):-

Compound (2) (21.0 mg, 31.4 μmol) was dissolved in 0.2 M acetate buffer (pH 5.3, 5 ml), and 1.97 ml of a 10^{-3}N hydrochloric acid solution (10.5 ml) containing $GdCl_3.6H_2O$ (93.3 mg, 0.251 mmol) were added thereto. The resultant mixture was shaken for 1 minute and concentrated. The residue was dissolved in water (2 ml) and subjected to high speed liquid chromatography for purification, followed by lyophilization to give Gd-DNS-etn-DTPA (20.3 mg). Yield, 79 %.

IR (KBr) cm^{-1}: SO_2-NH (1150, 1320), COO^- (1410, 1590), $C_{10}H_6$-N-$(CH_3)_2$ (2800), CH_2 (2950), CO-NH (3400).

FAB-MS (positive): M^+ (823 m/z), $(M + Na-H)^+$ (845 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{28}H_{37}N_6O_{11}S_1Gd_1.9H_2O$.

Example 9

Eu-DNS-etn-DTPA (complex) and La-DNS-etn-DTPA (complex):-

In the same manner as in Example 8 but using $EuCl_3.6H_2O$ or $LaCl_3.7H_2O$, the Eu or La complex with Compund (2) was prepared. There was thus obtained Eu-DNS-etn-DTPA (19.4 mg) in a yield of 77 % or La-DNS-etn-DTPA (14.4 mg) in a yield of 61 %.

Eu-DNS-etn-DTPA:-

IR (KBr) cm^{-1}: SO_2-NH (1150, 1330), COO^- (1410, 1600), $C_{10}H_6$-N-$(CH_3)_2$ (2800), CH_2 (2960), CO-NH (3420).

FAB-MS (negative):$(M-H)^-$ (817 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{28}H_{37}N_6O_{11}S_1Eu_1.7\frac{1}{2}H_2O$.

La-DNS-etn-DTPA:-

IR (KBr) cm^{-1}: SO_2-NH (1150, 1330), COO^- (1410, 1590), $C_{10}H_6$-N-$(CH_3)_2$ (2800), CH_2 (2950), CO-NH (3420).

FAB-MS (negative): $(M-H)^-$ (803 m/z).

The result of the elementary analysis corresponds to the empirical formula: $C_{28}H_{37}N_6O_{11}S_1La_1.8H_2O$.

Example 10

Radioactivity distribution of In-111-DNS-etn-DTPA and In-111-B-DNS-etn-DTPA in rats on intravenous injection:-

In-111-DNS-etn-DTPA or In-111-B-DNS-etn-DTPA was intravenously injected to Sprague-Dawley strain rats (female) at a dose of 25 μg/rat, and the rats were sacrificed one hour thereafter to take out various organs. The radioactivity in each organ was measured, and the results are shown in Table 2.

Table 2

Radioactivity distribution of In-111-DNS-etn-DTPA and In-111-B-DNS-etn-DTPA in rats (% injected dose/organ)

Organ	In-111-DNS-etn-DTPA	In-111-B-DNS-etn-DTPA
Liver	1.35	2.60
Bowel	68.01	91.86
Kidney	1.00	0.26
Urinary bladder	25.49	3.86
Blood (1 ml)	0.06	0.03
Others	7.97	2.74

From the above results, it is understood that In-111-B-DNS-etn-DTPA is an excellent radioactive diagnostic agent for examination of hepatobiliary tissues.

For comparison, the radioactivity distribution of In-111-DTPA (In-111 complex with DTPA), prepared as in Example 6 A, in rats on intravenous injection was determined as above. As the result, it was revealed that about 90 % of the radioactivity as given was excreted in urine within one hour after the administration.

It is thus understood that the excretion route of DTPA is changed to a hepatobiliary system by introduction of a dansyl group therein. In other words, a dansyl group may be said to be effective in construction of a hepatobiliary tissue-specific carrier.

Example 11

Radioactivity distribution of In-111-DNS-hxn-DTPA and In-111-B-DNS-hxn-DTPA in rats on intravenous injection:-

In-111-DNS-hxn-DTPA or In-111-B-DNS-hxn-DTPA was intravenously injected to Sprague-Dawley strain rats (female) at a dose of 25 μg/rat, and the rats were sacrificed one hour thereafter to take out various organs. The radioactivity in each organ was measured, and the results are shown in Table 3.

Table 3

Radioactivity distribution of In-111-DNS-hxn-DTPA and In-111-B-DNS-hxn-DTPA in rats (% injected dose/ organ)

Organ	In-111-DNS-hxn-DTPA	In-111-B-DNS-hxn-DTPA
Liver	0.51	1.82
Bowel	93.42	92.81
Kidney	0.05	0.12
Urinary bladder	5.35	0.46
Blood (1 ml)	0.01	0.05
Others	0.65	4.45

From the above results, it is understood that like In-111-B-DNS-etn-DTPA, tested In-111 complexes are both excellent diagnostic agents for hepatobiliary tissues.

Example 12

Imaging of rat hepatoma with In-111-B-DNS-etn-DTPA:-

In-111-B-DNS-etn-DTPA was intravenously injected into a hepatoma-transplanted Wister rat (male, tumor size: about 3 cm) at a dose of 50 ug/rat and the rat was kept in a cage for 70 hours. The rat was pronely fixed and subjected to imaging with a gamma-camera (manufactured by Toshiba, Ltd.). The scintigram thus obtained is shown in Fig. 1 of the accompanying drawings. While accumulation of In-111-B-DNS-etn-DTPA as in such major organs as liver and digestive organs was significant, the tumor was clearly imaged at the left shoulder of the rat.

From the above results, it is clear that In-111-B-DNS-etn-DTPA is taken up into hepatocellular carcinoma.

Example 13

A. Relaxation of Gd-DNS-etn-DTPA

Gd-DNS-etn-DTPA as obtained in Example 8 was dissolved in 10 mM acetate buffer (pH 5.5), and the relaxation time (T_1 and T_2, millisecond) was measured with regard to water proton by NMR (manufactured by Nihon Denshi; 270 MHz; 25° C). The results are shown in Table 4.

Table 4

Concentration (mM)	T_1	T_2
5.59	44	36
1.31	313	246
0.66	534	424
0	3260	1315

As understood from the above, Gd-DNS-etn-DTPA shows an excellent relaxation time. For instance, the T_1 and T_2 values of water were respectively shortened about 74 times and about 37 times at a concentration of 5.59 mM.

B. Pharmacodynamics on relaxation of Gd-DNS-etn-DTPA in mice:-

To each of ICR strain mice (female), a solution of Gd-DNS-etn-DTPA in 10 mM acetate buffer (pH 5.5) was administered at a dose of 0.02 mM/kg by injection into the tail vein. The mice were sacrificed by cutting their necks 1 minute, 1 hour and 6 hours after the administration. The proton relaxation value was measured on each organ in a test tube by NMR (270 MHz) at 25° C. Relaxation of T_1 and T_2 on each organ is shown in Table 5.

Table 5

Organ	Normal value		Time: After 1 min.		After 60 min.		After 360 min.	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
Liver	1040	17	776	16	745	17	1025	19
Heart	1484	29	1289	28	1408	26	1523	29
Kidney	1269	31	918	28	878	29	1196	33
Brain	1576	45	1556	-	1586	51	1613	50
Blood	1755	53	884	-	1713	67	1680	54

From the above results, it is understood that Gd-DNS-etn-DTPA is quickly taken up by liver, kidney and heart in mouse and excreted. Since the change of T_1 in liver and kidney with time is distinguished from that of T_1 in blood, the behavior of Gd-DNS-etn-DTPA in liver and kidney may be considered to be not originated from that in blood. Furthermore, Gd-DNS-etn-DTPA may be understood to afford an influence on the T_1 relaxation in a living body.

Claims

1. A chelating compound of the formula:

$$(R\text{-}NHOC\text{-}CH_2)_n\text{-}A\text{-}(CH_2COOH)_m \quad (I)$$

wherein R is an aromatic ring-containing organic group, A is a residue of an aminopolyacetic acid excluding acetic acid groups ($-CH_2COOH$) therefrom, m is an integer of at least two and n is an integer of 1 or 2, or its salt.

2. The compound according to claim 1, wherein the aromatic ring-containing organic group is aryl, ar-(lower)alkyl, arylsulfonyl, ar(lower)alkylsulfonyl, arylamino(lower)alkyl, ar(lower)alkylamino(lower)alkyl, arylsulfonylamino(lower)alkyl or ar(lower)alkylsulfonylamino(lower)alkyl, the aryl moiety being phenyl or naphthyl optionally substituted with lower alkyl, lower alkylamino or di(lower)alkylamino.

3. The compound according to claim 1, wherein the aromatic ring-containing organic group is dansyl.

4. The compound according to claim 1, wherein the aromatic ring-containing organic group is dansylamino(lower)alkyl.

5. The compound according to claim 1, wherein the aminopolyacetic acid comprises a hydrocarbon chain in a straight, branched or cylic form, at least two amino groups present in the hydrocarbon chain and/or at the terminal position and at least three acetic acid ($-CH_2COOH$) groups each attached to a nitrogen atom in said amino groups.

6. The compound according to claim 1, wherein the the aminopolyacetic acid is ethylenediamine-tetraacetic acid (EDTA), diethylenetriamine-pentaacetic acid (DTPA), trans-1,2-cyclohexadiamine-tetraacetic acid (CyDTA) or 1,4,7,10-tetraazacyclododecane-tetraacetic acid (DOTA).

7. A chelate compound, which comprises the compound according to any of claims 1 to 6, and a metallic element bonded thereto through a chelate bond.

8. The chelate compound according to claim 7, wherein the metallic element is a radioactive element.

9. The chelate compound according to claim 8, wherein the radioactive element is technetium-99m, indium-111, rhenium-186, rhenium-188 or yttrium-90.

10. The chelate compound according to claim 7, wherein the metallic element is a paramagnetic element.

11. The chelate compound according to claim 10, wherein the paramagnetic element is gadolinium.

12. A carrier for a metallic element which comprises the chelating compound according to any one of claims 1 to 6 having a chelate-forming property and a specificity to a hepatobiliary organ or tissue.

13. A diagnostic agent for hepatobiliary organs or tissues which comprises the chelate compound according to any of claims 7 to 11.

14. A therapeutic agent for hepatobiliary organs or tissues which comprises the chelate compound according to any of claims 7 to 11.

CLAIMS FOR THE FOLLOWING CONTRACTING STATE: ES

1. A process for the preparation of a chelating compound of the formula:

$$(R\text{-}NHOC\text{-}CH_2)_n\text{-}A\text{-}(CH_2COOH)_m \quad (I)$$

wherein R is an aromatic ring-containing organic group, A is a residue of an aminopolyacetic acid excluding acetic acid groups ($-CH_2COOH$) therefrom, m is an integer of at least two and n is an integer of 1 or 2, or its salt, which comprises reacting an aromatic ring-containing organic amine of the formula $R\text{-}NH_2$, wherein R is as defined above with an aminopolyacetic acid of the formula $(HOOCCH_2)_n\text{-}A\text{-}(CH_2COOH)_m$ wherein A, m and n are each as defined above in any reactive form in an inert solvent.

2. The process according to claim 1, wherein the aromatic ring-containing organic group is aryl, ar(lower)-alkyl, arylsulfonyl, ar(lower)alkylsulfonyl, arylamino-(lower) alkyl, ar(lower)alkylamino(lower)alkyl, arylsulfonylamino(lower)alkyl or ar(lower)alkylsulfonylamino(lower)alkyl, the aryl moiety being phenyl or naphthyl optionally substituted with lower alkyl, lower alkylamino or di(lower)-alkylamino.

3. The process according to claim 1, wherein the aromatic ring-containing organic group is dansyl.

4. The process according to claim 1, wherein the aromatic ring-containing organic group is dansylamino-(lower)alkyl.

5. The process according to claim 1, wherein the aminopolyacetic acid comprises a hydrocarbon chain in a straight, branched or cyclic form, at least two amino groups present in the hydrocarbon chain and/or at the terminal position and at least three acetic acid ($-CH_2COOH$) groups each attached to a nitrogen atom in said amino groups.

6. The process according to claim 1, wherein the aminopolyacetic acid is ethylenediamine-tetraacetic acid (EDTA), diethylenetriamine-pentaacetic acid (DTPA), trans-1,2-cyclohexadiamine-tetraacetic acid (CyDTA) or 1,4,7,10-tetraazacyclododecane-tetraacetic acid (DOTA).

7. A process for the preparation of a chelate compound, which comprises binding a compound prepared according to any of claims 1 to 6 and a metallic element through a chelate bond.

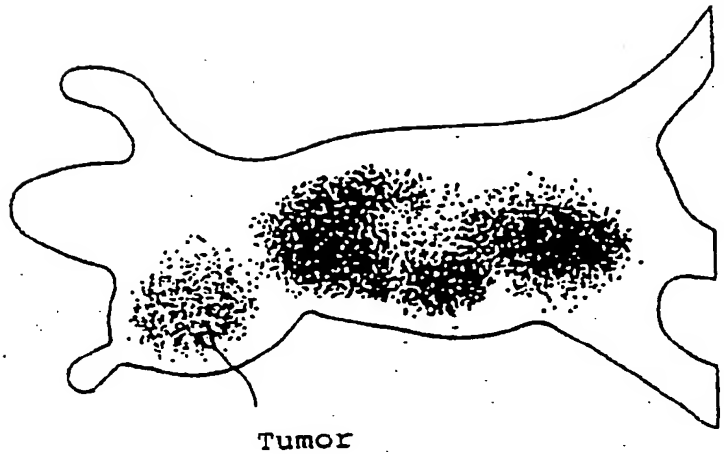
8. The process according to claim 7, wherein the metallic element is a radioactive element.

9. The process according to claim 8, wherein the radioactive element is technetium-99m, indium-111, rhenium-186, rhenium-188 or yttrium-90.

10. The process according to claim 7, wherein the metallic element is a paramagnetic element.

11. The process according to claim 10, wherein the paramagnetic element is gadolinium.

Fig. 1: Scintigram of hepatoma-transplanted rat using In-111-B-DNS-etn-DTPA (70 hours after administration)



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 451 824 A3**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **91105704.0**

(22) Date of filing: **10.04.91**

(51) Int. Cl.5: **C07C 311/36**, C07C 237/06, C07C 229/76, C07C 229/26, A61K 49/02, A61K 49/00, A61K 43/00

(30) Priority: **10.04.90 JP 94353/90**

(43) Date of publication of application: **16.10.91 Bulletin 91/42**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB IT LI LU NL SE**

(88) Date of deferred publication of the search report: **02.09.92 Bulletin 92/36**

(71) Applicant: **NIHON MEDI-PHYSICS CO., LTD.**
9-8, Rokutanji-cho, Nishinomiya
Hyogo(JP)

(72) Inventor: **Seri, Shigemi**
Sumitomo Kagaku Sodegaura Shataku 1-204
98 Daijuku
Sodegauramachi, Kimitsu-gun, Chiba(JP)
Inventor: **Yamauchi, Hirohiko**
Sumitomo Kagaku Anegasaki Shataku 825
1-9, Yushudainishi, Ichihara Chiba(JP)
Inventor: **Azuma, Makoto**
750, Saihiro
Ichihara, Chiba(JP)
Inventor: **Arata, Yoji**
c/o Urban Heights Kitasuna 1122
5-20-18, Kitasuna, Koto-ku, Tokyo(JP)

(74) Representative: **Vossius & Partner**
Siebertstrasse 4 P.O. Box 86 07 67
W-8000 München 80(DE)

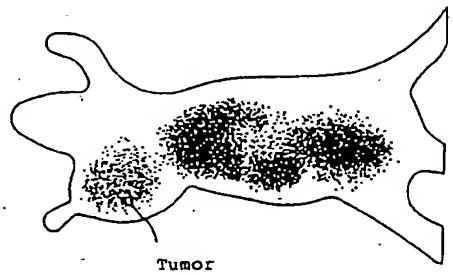
(54) Chelating compounds and their use.

(57) A chelating compound of the formula:

$(R\text{-}NHOC\text{-}CH_2)_n\text{-}A\text{-}(CH_2COOH)_m$ (I)

wherein R is an aromatic ring-containing organic group, A is a residue of an aminopolyacetic acid excluding acetic acid groups ($-CH_2COOH$) therefrom, m is an integer of at least two and n is an integer of 1 or 2, or its salt, which has a specificity to a hepatobiliary system so that a chelate compound formed between said chelating compound and a metallic element through a chelate bond is useful as a diagnostic or therapeutic agent for hepatobiliary organs and tissues.

Fig. 1: Scintigram of hepatoma-transplanted rat using In-111-B-DNS-etn-DTPA (70 hours after administration)



EP 0 451 824 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

DOCUMENTS CONSIDERED TO BE RELEVANT

EP 91105704.0

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	US - A - 4 316 883 (M. SCHRIJVER) * Claims; column 1, line 10 - column 2, line 6 * --	1,7-9, 12-14	C 07 C 311/36 C 07 C 237/06 C 07 C 229/76 C 07 C 229/26 A 61 K 49/02
X	EP - A - 0 107 292 (MERCK FROSST CANADA INC.) * Claims * --	1,7-9, 12,13	A 61 K 49/00 A 61 K 43/00
A	EP - A - 0 263 059 (SCHERING) * Claims * ----	1-13	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 C 311/00
C 07 C 237/00
A 61 K 49/00
A 61 K 43/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	25-06-1992	IRMLER

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)